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#19

United States Patent [19]

Brenner

[11] Patent Number: **5,604,097**
 [45] Date of Patent: **Feb. 18, 1997**

[54] **METHODS FOR SORTING
POLYNUCLEOTIDES USING
OLIGONUCLEOTIDE TAGS**

[75] Inventor: **Sydney Brenner**, Cambridge, England
 [73] Assignee: **Spectragen, Inc.**, Hayward, Calif.
 [21] Appl. No.: **358,810**
 [22] Filed: **Dec. 19, 1994**

Related U.S. Application Data

[63] ~~Continuation-in-part of Ser. No. 322,348, Oct. 13, 1994,
abandoned.~~
 [51] Int. Cl.⁶ **C12Q 1/68; C12N 15/10;
C07H 21/00**
 [52] U.S. Cl. **435/6; 536/25.4; 435/172.3**
 [58] Field of Search **435/6, 172.3, 320.1;
536/25.4**

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[57] ABSTRACT

The invention provides a method of tracking, identifying, and/or sorting classes or subpopulations of molecules by the use of oligonucleotide tags. Oligonucleotide tags of the invention each consist of a plurality of subunits 3 to 6 nucleotides in length selected from a minimally cross-hybridizing set. A subunit of a minimally cross-hybridizing set forms a duplex or triplex having two or more mismatches with the complement of any other subunit of the same set. The number of oligonucleotide tags available in a particular embodiment depends on the number of subunits per tag and on the length of the subunit. An important aspect of the invention is the use of the oligonucleotide tags for sorting polynucleotides by specifically hybridizing tags attached to the polynucleotides to their complements on solid phase supports. This embodiment provides a readily automated system for manipulating and sorting polynucleotides, particularly useful in large-scale parallel operations, such as large-scale DNA sequencing, mRNA fingerprinting, and the like, wherein many target polynucleotides or many segments of a single target polynucleotide are sequenced simultaneously.

31 Claims, 6 Drawing Sheets